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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Henry J. WINDLE et al.

Serial No.: 10/068,870 Examiner: To Be Assigned

Filed: February 11, 2002 Group Art Unit: To Be Assigned

For: CLOSTRIDIUM DIFFICILE VACINE

REQUEST FOR PRIORITY

Commissioner of Patents
Washington, D.C. 20231

Sir:

Please make of record the following attached certified copy(ies) of the corresponding

1.) Irish
2.) _____
3.) _____ application
Country

No.(s) of

1.) 2001/0137
2.) _____, filed
3.) 10/068,870
SN No.(s)

1.) 9 February 2001
2.) _____, the Priority
3.) 11 February 2002
Date(s)

which is hereby claimed under the provisions of 35 U.S. C. 119.

Respectfully submitted,

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Folio: 11472/P67635US0
Date: May 29, 2002

JCH-9/01-102

Patents Office
Government Buildings
Hebron Road
Kilkenny

I HEREBY CERTIFY that annexed hereto is a true copy of documents filed in connection with the following patent application:

Application No.	2001/0137
Date of Filing	9 February 2001
Applicant	THE PROVOST, FELLOWS AND SCHOLARS OF THE COLLEGE OF THE HOLY AND UNDIVIDED TRINITY OF QUEEN ELIZABETH, NEAR DUBLIN, a Registered charity of Ireland of College Green, Dublin 2, Ireland.

Dated this 23 day of January 2002.

An officer authorised by the
Controller of Patents, Designs and Trademarks.

FORM NO. 1

S010137

REQUEST FOR THE GRANT OF A PATENT

PATENTS ACT, 1992

The Applicant(s) named herein hereby request(s)

__X__ the grant of a patent under Part II of the Act

_____ the grant of a short-term patent under Part III of the Act

on the basis of the information furnished hereunder.

1. Applicant(s)

Name: The Provost, Fellows and Scholars of the College of the Holy and Undivided Trinity of Queen Elizabeth, Near Dublin

Address: College Green
Dublin 2
Ireland

Description/Nationality

A Registered charity of Ireland

2. Title of Invention

"A vaccine"

3. Declaration of Priority on basis of previously filed application(s) for same invention (Sections 25 & 26)

Previous filing date	Country in or for which filed	Filing No.

4. Identification of Inventor(s)
Name(s) of person(s) believed
by Applicants(s) to be the inventor(s)

Name: Rachael Doyle, an Irish citizen of
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Name: Henry J Windle, an Irish citizen of
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Name: James Bernard Walsh, an Irish citizen of
Address: 3 Ardlui Park, Blackrock, County Dublin, Ireland

5. Statement of right to be granted a patent (Section 17(2) (b)

The applicant derives the right to the invention by virtue of a Deed of Assignment dated December 22, 2000.

6. Items accompanying this Request – tick as appropriate

(i) __X__ prescribed filing fee (£100.00)

(ii) __X__ specification containing a description and claims

____ specification containing a description only

__X__ Drawings referred to in description or claims

(iii) ____ An abstract

(iv) ____ Copy of previous application (s) whose priority is claimed

(v) ____ Translation of previous application whose priority is claimed

(vi) __X__ Authorisation of Agent (this may be given at 8 below if this Request is signed by the Applicant (s)

7. Divisional Application (s)

The following information is applicable to the present application which is made under Section 24 –

Earlier Application No: .....

Filing Date: .....

8. Agent

The following is authorised to act as agent in all proceedings connected with the obtaining of a patent to which this request relates and in relation to any patent granted -

Name	Address
John A. O'Brien & Associates	The address recorded for the time being in the Register of Patent Agents, and currently Third Floor, Duncairn House, 14 Carysfort Avenue, Blackrock, Co. Dublin, Ireland.

9. Address for Service (if different from that at 8)

As above

Signed _____ JOHN A. O'BRIEN & ASSOCIATES

Date February 9, 2001

"A vaccine"

Introduction

The invention relates to proteins or derivatives or fragments thereof obtained from *Clostridium difficile*. In particular the invention relates to the use of these proteins as vaccines to provide immunological protection against *C. difficile* infection.

Background

Clostridium difficile is a common nosocomial pathogen and a major cause of morbidity and mortality among hospitalised patients throughout the world [1]. Outbreaks of *C. difficile* have necessitated ward and partial hospital closure. With the increasing elderly population and the changing demographics of the population, *C. difficile* is set to become a major problem in the 21st century. The spectrum of *C. difficile* diseases range from asymptomatic carriage to mild diarrhoea to fulminant pseudomembranous colitis. Host factors rather than bacterial factors appear to determine the response to *C. difficile* [2-4].

Reports indicate that hypogammaglobulinaemia in children appears to predispose to the development of disease due to *C. difficile* and that therapy with intravenously administered gamma globulin can be associated with the clinical resolution of chronic relapsing colitis due to *C. difficile* disease [5,6].

In a study of 16 patients with *C. difficile* diarrhoea (CDD), 8 patients had serum IgG that was reactive with a surface protein of 36 KDa from their infecting *C. difficile* strain, and patients showed a convalescent increase in IgG reactivity to this protein [7]. This protein has been partially characterised, but its function is unknown [8]. A study by Mulligan et al. found elevated levels of

immunoglobulins reactive with *C. difficile* in asymptomatic carriers as opposed to symptomatic patients [9]. Recently it has been shown that patients who became colonised with *C. difficile* who had relatively low levels of serum IgG antibody against toxin A had a much greater risk of developing *C. difficile* diarrhoea [10].

It is clear that any advance in the understanding of *C. difficile* disease and methods of preventing or treating *C. difficile* diarrhoea (CDD) and other related diseases will be of huge therapeutic potential.

Statements of Invention

According to the invention there is provided a protein capable of producing an immune response in individuals who recover from *C. difficile* infection.

The invention also provides a *C. difficile* protein comprising SEQ ID no. 1.

The invention also provides a *C. difficile* protein comprising SEQ ID no. 2.

The invention also provides a *C. difficile* protein comprising SEQ ID no. 3.

The invention also provides a *C. difficile* protein comprising SEQ ID no. 4.

Preferably the *C. difficile* protein has a molecular weight of from 30 to 35kDa, most preferably having a molecular weight of approximately 31kDa or a molecular weight of approximately 33kDa.

The invention further provides a derivative or fragment or mutant of a protein of the invention.

The invention provides a vaccine comprising a protein capable of producing an immune response in individuals who recover from *C. difficile* infection.

In one embodiment of the invention the vaccine comprises a *C. difficile* protein or a derivative or fragment or mutant thereof capable of producing an immune response in individuals who recover from *C. difficile* infection.

Preferably the vaccine comprises a protein of the invention in combination with a *C. difficile* sub-unit.

Most preferably the vaccine comprises one or more pharmacologically suitable adjuvant(s). Ideally the vaccine includes at least one other pharmaceutical product such as an antibiotic. The antibiotic may be metronidazole or vancomycin.

Preferably the vaccine is in a form for oral, intranasal, intravenous or intramuscular administration.

The invention also provides a method of inducing protective antibodies against *C. difficile* in animals including humans, comprising the step of administering to an animal a protein of the invention or a derivative or fragment thereof.

The invention also provides antibodies whenever produced by such a method.

One embodiment of the invention provides antibodies for use in passive immunotherapy for established *C. difficile* infection.

Another embodiment of the invention provides use of an antibody of the invention in the preparation of a medicament for the eradication of *C. difficile* associated disease.

A further embodiment of the invention provides use of a *C. difficile* protein of the invention or a derivative or fragment or mutant thereof in the preparation of a medicament for the prophylaxis and/or treatment of *C. difficile* associated disease.

Brief Description of the Drawings

The invention will more clearly understood from the following description thereof given by way of example only with reference to the accompanying drawings, in which:-

Fig. 1a is a Western blot showing the recognition of the 33 kDa antigen in serum obtained from a convalescent individual (lane 4); and

Fig. 1b is a Western blot showing the recognition of the 31 kDa antigen in serum obtained from convalescent individuals (lanes 6 and 7);

Detailed Description

We have found that two antigenic proteins having a molecular weight in the range of 30 to 35 kDa associated with *C. difficile* induce a strong immune response in individuals who recover from *C. difficile* infection. The proteins are therefore ideal candidates for the preparation of vaccines against *C. difficile.*

We have purified a 33 kDa and a 31 kDa protein for use as a therapeutic immunogen for the eradication of *C. difficile* infection.

Antibodies to the 33 kDa and the 31 kDa proteins were developed which may be used as passive immunotherapy for established *C. difficile* infection.

Such purified proteins may be used in combination with other *C. difficile* sub-units as a combined vaccine against *C. difficile.*

It will be understood that other purified proteins of *C. difficile* to which constitutive antibodies are detected in individuals recovering from *C. difficile* infection are also covered under the scope of the present invention.

Western blotting and enhanced chemiluminesence was used to demonstrate that patients with *C. difficile* infection who recover from the infection develop acute phase antibody responses to previously unrecognised antigens associated with *C. difficile* and that these antibodies persist during convalescence (Figs. 1a, b). Characterisation of the antigens recognised by these antibodies was performed and the molecular weight and the sequence of the 20 amino acid residues at the N-terminus of each antigen was determined.

A deposit of the 33kDa *Clostridium difficile* protein was made at the NCIMB on January 29, 2001 and accorded the accession number NCIMB 41080.

A deposit of 31kDa *Clostridium difficile* protein was made at the NCIMB on January 29, 2001 and accorded the accession number NCIMB 41081.

The proteins of the present invention have the following sequences. A major and minor signal was detected for both proteins.

<u>33kDa Protein</u>

Sequence ID No. 1: DKTKVETADQGYTVVQSKYK (major signal)

Sequence ID No. 2: MXILGXGGTRYEHPRINRK (minor signal)

31kDa Protein

Sequence ID No. 3 ATTGTQGYTVVKNDGKKAVK (major signal)
Sequence ID No. 4 MKIMVEVSKDADQPIMNRSI (minor signal)

The invention will be more clearly understood from the following examples.

Methods and Materials

Western blotting

Proteins from SDS-PAGE gels were electroblotted (0.8mA/cm2 for 1 h) to PVDF membrane using a semi-dry blotting apparatus (Atto). Primary antibodies (human serum: 1/50 – 1/100 dilution) were detected using a 1/5000 dilution of anti-human IgG (horse radish peroxidase-conjugated) in combination with enhanced chemiluminesence (ECL). Blots were washed in phosphate buffered saline (pH 7.5) containing fat-free dried skim milk (5%, W/v) and Tween-2- (0.05%, v/v). [Blots were exposed to Kodak X-OMAT film for various periods of time and developed].

Partial purification of the 33 kDa and the 31 kDa proteins

The antigens were partially purified from *C. difficile* based on their molecular weight using preparative continuous-elution SDS-PAGE on a model 491 Prep-Cell (Bio-Rad). The appropriate antigens were subsequently identified on Western blots probed with serum obtained from individuals who recovered from *C. difficile* infection.

Clinical Study

Examination of sequential antibody responses to *C. difficile* among elderly patients who developed the disease was carried out. The study was based on the hypothesis that the host immune response influenced the development of *Clostridium difficile* disease. In particular we determined that a particular pattern of immune response to *C. difficile* antigens correlated with the outcome of CDD.

Method

Serum was collected from over 300 patients and of these 30 patients developed CDD. The infecting strain (homologous strain) was grown from each patient. Strains of *C. difficile* were typed at the Anaerobic Reference Laboratory, Wales [11]. The most common strains isolated were PCR type 1 (n = 15) which is the most common type causing epidemics and PCR 12 (n = 5) which is also a common hospital strain. Pre-infection serum samples were obtained from patients. Acute phase sera were then collected from patients who developed *C. difficile* disease. Convalescent sera were collected from patients who recovered. Protein extracts of patients' infecting *C. difficile* strain were probed with the patients sera using Western blotting. IgG responses to the antigens were examined. Overall 5 patients made a full recovery and new antibody responses to previously unrecognised antigens were evident in 4 of these patients. Three of these patients had *C. difficile* belonging to PCR type 1 and one patient had *C. difficile* PCR type 12. These patients developed an acute phase antibody response to previously unrecognised *C. difficile* antigens which persisted during convalescence (Fig. 1a, b). Antibody recognition of these antigens was associated with recovery and may consequently represent candidate vaccine antigens. These antibody responses have also been found in some controls in the same ward who were also on antibiotics but who did not develop CDD.

The immunodominant protein which was associated with a positive outcome from PCR 12 was identified and purified using preparative SDS-PAGE. The N-terminal region of the protein was sequenced using an Applied Biosystems Procise Sequencer (sequence 1,2).

The antigen which was associated with a protective antibody response from the PCR 1 strain was identified and the N-terminus determined (sequence 3,4).

There are a number of papers dealing with vaccination strategies using animal models (hamster) of *C. difficile* infection (12, 13). To date it does not appear that any human studies have been carried out.

The invention is not limited to the embodiments hereinbefore described which may be varied in detail.

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Claims

1. A protein capable of producing an immune response in individuals who recover from *C. difficile* infection.

2. A *C. difficile* protein comprising SEQ ID no. 1.

3. A *C. difficile* protein comprising SEQ ID no. 2.

4. A *C. difficile* protein comprising SEQ ID no. 3.

5. A *C. difficile* protein comprising SEQ ID no. 4.

6. A *C. difficile* protein having a molecular weight of from 30 to 35kDa.

7. A *C. difficile* protein having a molecular weight of approximately 31kDa.

8. A *C. difficile* protein having a molecular weight of approximately 33kDa.

9. A derivative or fragment or mutant of a protein as claimed in any of claims 1 to 8.

10. A vaccine comprising a protein capable of producing an immune response in individuals who recover from *C. difficile* infection.

11. A vaccine comprising a *C. difficile* protein capable of producing an immune response in individuals who recover from *C. difficile* infection.

12. A vaccine comprising a *C. difficile* protein as claimed in any of claims 2 to 8 or a derivative or fragment or mutant thereof.

13. A vaccine comprising a protein as claimed in any of claims 1 to 8 in combination with a *C. difficile* sub-unit.

14. A vaccine as claimed in any of claims 10 to 13 comprising one or more pharmacologically suitable adjuvant(s).

15. A vaccine as claimed in any of claims 10 to 13 including at least one other pharmaceutical product.

16. A vaccine as claimed in any of claims 15 wherein the pharmaceutical product is an antibiotic.

17. A vaccine as claimed in claim 15 or 16 wherein the antibiotic is metronidazole or vancomycin.

18. A vaccine as claimed in any of claims 10 to 17 in a form for oral, intranasal, intravenous or intramuscular administration.

19. A method of inducing protective antibodies against *C. difficile* in animals including humans, comprising the step of administering a protein or derivative or fragment thereof as claimed in any of claims 1 to 9.

20. Antibodies whenever produced by a method as claimed in claim 19.

21. Antibodies as claimed in claim 20 for use in passive immunotherapy for established *C. difficile* infection.

22. Use of an antibody as claimed in claim 20 in the preparation of a medicament for the eradication of *C. difficile* associated disease.

23. Use of a *C. difficile* protein as claimed in any of claims 1 to 8 or a derivative or fragment or mutant thereof in the preparation of a medicament for the prophylaxis and/or treatment of *C. difficile* associated disease.

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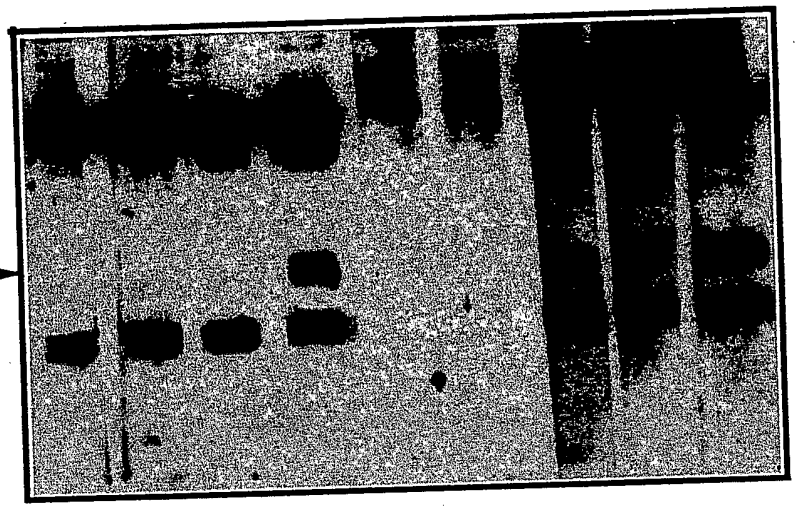
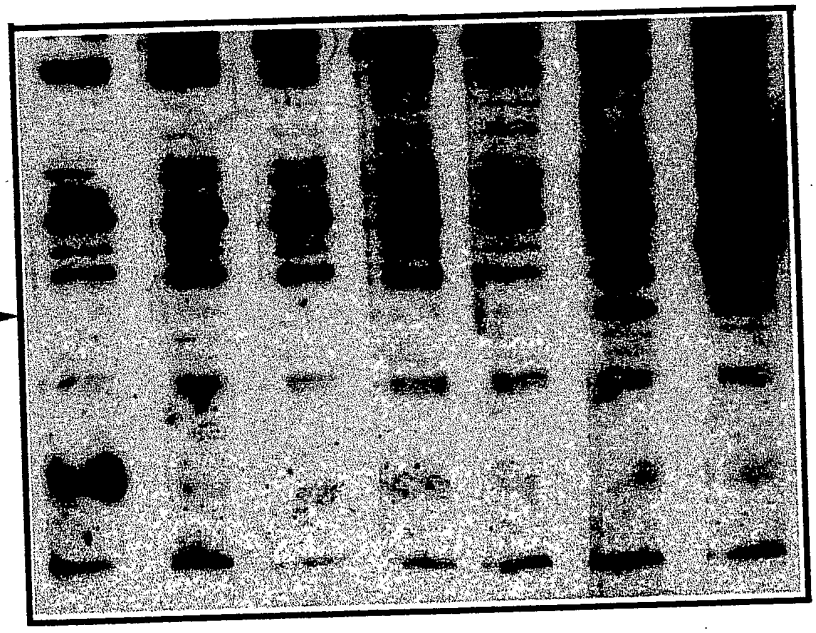
A



Key: 1 = pre-infection 2= early acute. 3 = late acute 4 = convalescent

5 = control 1 6 = control 2 7 = control 3

B



Key: 1 = Pre-infection 2-5 = acute 6-7 = convalescent

Fig. 1